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Evaluation of Acute Toxicity Study of Ethanolic Extract of Koelreuteria Paniculata Laxm in Albino Wistar Rats

Sonia Ranawat[1], Monika Gariya[2], Akhilesh Nautiyal[*3], Shagil Saltanat[4], Tripti Padalia[5], Varnika[6]

[1]Assistant Professor, School of Pharmacy, Maya Devi University, Dehradun, Uttarakhand, India

[2]Assistant Professor, Guru Ram Das (PG) Institute of Management and Technology, Dehradun, Uttarakhand, India

[*3]Assistant Professor, Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, Uttarakhand, India

[4]Assistant Professor, Sai Institute of Medical Sciences, Jaspur, Udham Singh Nagar, Uttarakhand, India

[5]Assistant Professor, School of Pharmaceutical Sciences, Amrapali University, Nainital, Uttarakhand, India

[6]Assistant Professor, Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Uttarakhand, India

[*]**Corresponding author:**

Akhilesh Nautiyal

Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun

Email id: akhilhealthcare373@gmail.com

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ABSTRACT

Koelreuteria paniculata, also named as golden rain tree, indigenous to China and other Asian countries. The current study examines its potential benefits in treating a variety of pharmacological disorders, including genotoxicity, antihypertensive, antioxidant, oxidative stress, antimalarial, and antineoplastic conditions. According to OECD guideline 423, acute toxicity studies examine the efficacious of the medicinal plant (*Koelreuteria paniculata*) and are helpful for further in vivo and in vitro investigations of other pharmacological illnesses.

Keywords: *OECD, Genotoxicity, Acute Toxicity Studies, Antihypertensive, Antineoplastic, Antioxidant.*

1. INTRODUCTION

Koelreuteria paniculata, often known as "pride of India," "golden rain tree," or "varnish tree". The soap berry, or Sapindaceae, family, includes the genus ***Koelreuteria Paniculata***.The tree is native to Korea and Northern China. Species of the Golden Rain tree has a brief lifespan.

It grows quickly and can withstand a variety of soil types and moisture conditions. The first time ***Koelreuteria paniculata*** was seen in a riparian habitat—that is, in the extent betwixt the river Rupal's base bricks in boom blocks of river rupel in boom—was in 2014.[1]

By 2017, second individual was discovered in Kruibeke's Scheldt River in the same environment. Occasionally a multi-branched shrub or a deciduous tree, ***Koelreuteria paniculata*** has thick, brownish-gray to blackish-graybark. The oval-shaped leaves of *Koelreuteria paniculata* are divided into 7–15 leaf segments, each measuring roughly 7–10 cm in length. The leaves of this plant appear bronze in the spring and transition in the fall to yellow-orange. Chymes are the nuts that develop on branches. [2]

June and July are when the tree's yellowish blossoms bloom. Subsequently flowering, fruit, which is around 4 cm in size, resemble to bladder. The fruit has a black seed and turns from green to metallic brown in color. A tree's fruit lasts for a long drawn-out time. The oval-shaped fruits of ***Koelreuteria paniculata*** feature tangle exteriors and petals with acuminate apexes.[3]

Koelreuteria paniculata planting is characterized by its ease of use, light preference, cold tolerance, and drought tolerance. They require calcium-based soil that has weathered from limestone in order to grow. The plant has a high degree of flexibility.

In China, ***Koelreuteria paniculata*** begins to sprout in late spring and falls in early autumn. In the seaward reaches of the Yangtze and Yellow River basins, it grows more slowly.[4]

Early summer or the first week of July is when the tree's panicles, which are enfold with yellow flowers, appear. Some of these panicles have the shape of red corollas. The big, pinnate leaves turn a deep yellow in the fall before they fall. Fruit-bearing seeds are encased in lantern-shaped bracts that are strung like beads. It serves as courtyard decoration and greening. The seed of ***Koelreuteria paniculata*** is used to extract essential oil, while the woody portion is utilized to produce tiny utensils. For the culture within its region, the tree's leaves are utilized as a black dye.[5] Antioxidant and anti-tumor properties are among the many therapeutic benefits and phytochemistry of ***Koelreuteria paniculata***. The flavonoids and galloyl derivatives found in seeds indicate their potential application as insecticidal agents. The infusion of the flowers works well as an ocular rinse for the eyes.[6]

Koelreuteria paniculata variations vary according to their look and location. For example, in Northern China and Korea, the leaves are categorized in a individual pinnate pattern, whereas in Western China, the leaves are placed in a bi-pinnate pattern. Throughout the world, especially in Bulgaria, this plant species has been utilized to landscape parks, roadsides, and industrial sites. Overcoming the contamination caused by heavy metals has been impressive.[7]

The physiologically active extract obtained from different parts of ***Koelreuteria paniculata*** includes terpenoids, phenolic as well as saponins compounds. Using different extracts that are responsible for the plant's pharmacological actions, phytochemical screening.[8]

From the seedling of ***Koelreuteria paniculata***, a new variation known as "Jinluan 2" was discovered. In the spring, the petioles, tender branches, and leaves all exhibit an orange-red color.When fully grown, the hue changes yellow-green in the summer. The grafting technique-based propagation method is appropriate for cultivation in China's Northern and Central Regions.[9]

Fig1: Koelreuteria paniculata

Habitat: The plant grows between 30 and 40 feet tall, reaching a mature spread of about 35 feet.

Leaves: The leaves are green, 7–15 toothed, and 1-4 inches long.

Bark and twigs: In a zigzag pattern, with lenticels and scars

Fruits and Flowers: The flowers have a shiny, gold hue.

Papery fruit that is 12–18 inches long and contains black seeds develops.

TAXONOMY

Koelreuteria apiculata

VERNACULAR NAME: Golden Rain Tree or Pride of India

Taxonomical Hierarchy:

Kingdom	Plantae
Subkingdom	Viliplantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Sapindales
Family	Sapindaceae / Soapberries
Genus	Koelreuteria
Species	Koelreuteriapaniculatalaxm

Chemical components present throughout the plant:

Throughout the entire plant, many gallate derivatives, cyanolipids, and flavonoids have been identified. Ethyl p-trigallate, 3"-O-galloyl-4'-O-galloyl-gallic acid, 3'-galloylquercitin, galloylepicatechin, and other galloyl derivatives methyl p-digallate, ethyl-p-heptagallate, methyl-m digallate, p-digalloyl acid, m-digalloyl acid, galloyl-gallic acid, and Flavonoids include hyparin, catechin, isohamnetin, kaempferol-3-O-alpha-L-rhamnoside, quercitin-3-O-pyranosidase, quercitin-3-O-pyranosidase, quercitin, and isohamnetin. By comparing the known compounds' spectra, chemical characteristics, and physical attributes with those documented in the literature, their structures were determined.[10]

Pharmacological Activities of the Medicinal Plant *Koelreuteria paniculata:*

Antioxidant: Based on chemical and spectral evidence, the two novel flavonol glycosides identified in this study were extracted from dried *Koelreuteria paniculata* leaves and identified as (6,8-dihydroxy-afzelin) and afzelin 3"-O-gallate. Nine polyphenolic metabolites are known to exist, eight of which were isolated from this species for the first time. These glycosides have strong antioxidant properties, according to reports. Secondary metabolites or plant extracts have been used in phytotherapeutic medications as genoprotective agents and antioxidants to stave against a variety of illnesses.

Koelreuteria paniculata flower extract was shown to contain three carotenoid compounds: lutein, lycopene, and beta-carotene. The Trolox equivalent antioxidant capacity (TEAC) assay (also called ABTS assay) was carried out, according to Thiapong et al. The antioxidant content of *K. paniculata* flower extract was investigated in vitro, and the carotenoid fraction was separated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay. The calculated percentage of *K.Paniculata* carotenoid extract, which was contained in 1.41g, was 2.82%.[11]

Genotoxicity: Secondary metabolites or plant extracts have been used in phytotherapeutic medications as genoprotective to ward off a variety of illnesses. Genotoxicity defined the detrimental alterations in gene sequences brought on by the presence of genotoxins. These dangerous genotoxins interfere with chromosomal abbreviations, genetic mutations, and recombination in addition to impairing gene sequence. The genoprotective potential was examined using the extract and fraction of *Koelreuteria paniculata* laxm. Both the leaves of the K. paniculata extract and its fraction (KPE & KPF) showed the DNA protective effect in the thymus portion of the calf/pUC18 DNA protection studies. However, the pUC18 DNA breaks and loses its band when the plasmid is bring into the reagents (Fenton's). [12]

Antiproliferative: The extract of ethanol from the two tumor cell lines, HT29 and PC3, were used to test the antiproliferative properties of various parts of *Koelreuteria paniculata*. The cell line is found in the colon part of human is called as HT29 cell line.
Researcher used the term Adenocarcinoma a type of glandular cancer in the colon and has a large number of mature intestinal cells. The floral extract's positive antiproliferative action on HT-29 was demonstrated (IC50-23.63 μg /ml), with less noticeable activity in the PC3 case (IC50-58.76 microgram/ml). The extract of the leaves is less sensitive in PC3 prostate tumor-initiating cells, (80.56 microgram/ml), it exhibits a comparable effect on HT29 (IC50-23.63 μg /ml) cell lines. *Koelreuteria paniculata* bark extract was shown to be weakly sensitive, with a weak inhibitory impact on PC3 cancer cell lines (IC50-182.8μg /mL) and HT29 malignant cell lines (IC50-339.4 μg/mL), respectively. For HT29 as well as PC3 cell lines, it was evident that the bark extract of *Koelreuteria paniculata* had dose-dependent antiproliferative effects. Cell growth was impacted by the flower and leaf extract at doziness concentrations, while the similar result was observed at greater concentrations of 60 mg/mL.[13]

Antibacterial: Bark extract of koelreuteria paniculata is highly potent in resist to Gram-positive bacteria(Bacillus cereus and Bacillus subtilis) and responsible for the antibacterial activity. Higher concentrations of bark extract were found to be more

efficient against Gram-negative bacteria, such as Proteus vulgaris and Pseudomonas aeruginosa. Extract of *Koelreuteria paniculata* flower is highly effective as bark extract to resist the both types of bacteria(Gram-positive and Gram-negative bacteria), such as P. vulgaris (10 mm IZ), B. subtilis (14 mm IZ), and B. cereus (14 mm IZ).[14]

Antimicrobial: The capacity of the methanolic extract of K. paniculata leaves to stop the growth of S. aureus and B. subtilis bacteria.
Zazharskyi et al. looked into the antimicrobial properties of an ethanolic extract of ***K. paniculata*** leaves, which contains According to E. Ham et al., neryl acetate, which gives the bark extract of ***Koelreuteria paniculata*** its antibacterial properties, is present in the extract. Antibacterial activity was found in Bacillus subtilis, Pseudomonas aeruginosa,Bacillus cereus, Escherichia coli.[15]

Antimalarial: The antimalarial properties of methyl gallate and ethyl gallate were determined from these isolated molecules. The chloroquine-resistant (W2) and chloroquine-sensitive (D6) were both susceptible to the antimalarial action. ***Plasmodium falciparum*** is a protozoan that affects chloroquine-sensitive (D6) individuals with an IC50 of 1.28 μg /mL. For chloroquine-resistant (W2) individuals, the IC50 values were 0.77 and 1.85 μg / /mL, respectively. According to the IC50 value, ***Koelreuteria paniculata*** leaf extract exhibits antimalarial activity against *Plasmodium falciparum* protozoa. Eleven known compounds and three novel triterpenoid saponins, Paniculatosoid A, B, and C, were assessed and their antimalarial, antifungal, and antileishmanial properties documented. The ability of the antimalarial to inhibit chloroquine-sensitive (D6).

IC50 values were 6.46 and 6.95 μM for *Plasmodium falciparum* protozoa and 9.34 and 4.18 μM for chloroquine-resistant (W2) Plasmodium falciparum protozoa. [16]

Anti-alzheimer: Neurodegenerative diseases are long-lasting conditions marked by the gradual and steady deterioration of nerve cells in both the brain and spinal cord. Conditions such as Alzheimer's, Parkinson's, and Huntington's significantly contribute to the decline in cognitive functions, movement, and behavior that often comes with aging. [17]

Compounds 1–5 of the five novel barrigenol-type triterpenoids found in ***Koelreuteria paniculata*** Laxm seeds were identified using spectroscopy. The identification of Compound 1 was Barrigenol 16-O-2-methylbutanoyl-A2.

3-O-[beta galactopyranosyl (1-2)-alpha-L-arabinofuranosyl (1-3)(6-O-methyl)Compound 2 was determined to be beta Dglucuronopy-ranosyl-28-O-2-methyl butanoyl-A2 barrigenol. Compound 3 was found to be 3-O-[beta galactopyranosyl(1-2)]Barrigenol-(6-O-methyl) butanoyl-A228-O-2-methyl-beta D-glucuronopy-ranosyl. Alpha-L-arabinofuranosyl (3-O-)(1-3)The chemical 4.A2 barrigenol, also known as beta Dglucuronopyranosyl-22-O-2-methyl butanoyl, was found to be beta galactopyranosyl(1-2)-(6-O-methyl). Compound 5 was found to be 3-O-[betaD-galactopyranosy (1-2)].

Butanoyl-A2 barrigenol, 6-O-methyl)-beta D-glucuronopyranosyl-22-O-2-methyl. Five new barrigenoltriterpenoids found in the ethanolic extract of ***K. paniculata*** seed reduce the swimming ability of treated mice, assist in the depletion of the amyloid plques by lowering the hyperphosphorylation of tau protein caused by okadaic acid-treated mice, and also regulate glycogen synthase kinase, which is beneficial for its anti-alzheimer activity.[18]

Stress Releiver: To find out how ***Koelreuteria paniculata*** responded to varying Mn concentrations in terms of photosynthetic and antioxidant enzyme activity, a hypnotic test was performed. The outcome demonstrates that Mn stress reduces plant biomass and chlorophyll content.

Ascorbate peroxidase (APX), catalase activity (CAT), peroxidase (POD), malondialdehyde (MDA), and superoxide dismutase (SOD) levels were significantly elevated following Mn therapy, indicating that an antioxidant system might lessen oxidative stress under mild to severe Mn stress.[19]

Antineoplastic Carotenoids confined from the flower of the plant ***Koelreuteria paniculata*** laxm were tested for antineoplastic activity in BJ, HepG2, and MDA-MB-231 cell lines, and the DNA-shielding qualities of its fraction were investigated in calf thymus DNA.. Cell viability testing was carried out using the MTT assay technique.HepG2 was shown to be the most sensitive cell line, with an IC50 of 459.9 micrograms/ml. MDA-MB-231 showed a mild inhibitory effect on cell line proliferation (IC50=522.2 μg/ml).[20]

Oxidative Stress: Oxidative stress brought on by xanthine oxidase (XOD) in live tissue. The enzyme Xanthine Oxidase (XOD), which is necessary to break down purine nucleotides and create uricacid, is also the cause of gout, a prevalent kind of arthritis. This was prevented by the tannins.

Similar to tannins, certain galloylated flavonoids have been shown to have an inhibitory effect on XOD and to function as XOD inhibitors.The leaf extract of *Koelreuteria paniculata*laxm was shown to contain quercitin, a galloylated flavonoid that is significant in reducing Xanthine Oxidase synthesis and preventing the onset of gout.

These galloyalated flavonoids exhibited inhibitory activity with an IC50 value of 1.9-3.5*10M.These results imply that the compactness and interrelation between the galloyl and aglycon groups, as well as the discrepancies between them, demonstrate the inhibitory action.[21]

Myopia and conjunctivitis: Linoleic acid, lupinol, and retinal aldehyde are the main ingredients of the ethanolic and

methanolic benzene/ethanol extract of Koelreuteria paniculata roots from these solvents. Retinal aldehyde is a action molecule that convey with the retina's sensory cells based on signals. This active component treats myopia and conjunctivitis by acting on the retina.[22]

Antihypertensive: Linoleic acid is present in the ethanolic and methanolic root extracts of *Koelreuteria paniculata*. Linoleic acid is the name given to the unsaturated form of free fatty acid. A high level of linoleic acid in the blood serum causes CVS and CHS to develop. By constricting blood vessels and improving blood microcirculation, this plant's active ingredient lowers cholesterol levels and decreases hypertension, or high blood pressure. By constricting the blood vessels and acting as a scavenger of blood vessels, linoleic acid lowers the buildup of cholesterol in the blood and prevents hypercholestrolemia and hypertension.[23]

2. MATERIALS AND METHODS

Plant Collection and its Identification:

The plant was procured and Identified from Forest Research Institute (FRI) and authentified from Botanical Survey of India (BSI) which was certified with a given batch number: BSI/NRC/Tech/Herb.(Ident.)/2022-23/89.

Procurement of Experimental Animals:

150g-200g Wistar rats of either sex were procured from Shri Guru Ram Rai University, Dehradun.

Housing Condition:

The animals were kept in a standard laboratory setting with a temperature of 22°C to 20°C, 12 hours a day, 12 hours at night, and a humidity of 50+% regular laboratory diet and unrestricted access to water. All Protocols were followed the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines under Institutional Animal Ethical Committee (IAEC).

Preparation of ethanolic extract of Koelreuteria paniculata leaves(cold maceration):

The leaves were cleaned and allowed to dry for three to four days in the shade. The leaves were prepared into a coarse powder using a grinder. In a conical flask, the coarse powder was combined with 100% ethanol. A glass rod used to completely stir the mixture. For 72 hours, the conical flask was shaken intermittently. After that, the mixture was filtered using Whattman No. 1 filter paper and a muslin towel. The filtrate were concentrated at 40°C using a rotary evaporator, and the residue were stored in a refrigerator until it is needed again.[23]

OECD Guidelines for Acute Toxicity Studies:

The up-down testing method was initially defined by Dixon and Mood. In 1985, Bruce proposed the use of an up-down approach to assess the acute toxicity of a substance. Reducing the number of animals required to assess a chemical's acute oral toxicity is possible with the testing procedure described in the guideline.[24]

Experimental Study:

The oral acute toxicity study of the aqueous extract of *Koelreuteria paniculata* were carried out using the 'Up-and-Down' method of testing in albino rats at single doses of 175, 500, and 2000 mg/kg in accordance with the Organization For Economic Development (OECD) guideline no. 423.[25]

Assignment of animals: Six Albino rats were used for each dose level in the study and identified by using a marked.

Housing and Diet: Animals housed in polypropylene cages under maintain temperature and humidity (23*C).Survival of animals for 12 hr in dark, and 12 hr light condition. Animal were picked at a time, weighed and dosed with the equivalent volume of extract dissolved in distilled water.

Administration of doses: The extract was administered orally using gastric feeding tube, at different dose level of 175,500,2000 mg/kg upto 14 days. Each animal was observed after dosing for the first 5 minutes for signs of regurgitation and kept in a metallic cage.

Observation Period: Each animal were observed every 15 minutes in the first 4 hour after dosing, every 30 min for 6 hour and daily for 48 hour for behavioural signs of toxicity (changes in skin, hair, eyes, mucous membrane, and respiratory, circulatory , autonomic and central nervous system, motor activity, convulsion, tremors, salivation, diarrhoea, lethargy or sleep). According to specifications of the OECD, animals were monitored for a total of 14 days for the long-term possible lethal outcome. The body weights of the animals were measured on days 1, 7, and 14.[26]

Table 1 : Experimental Design for Acute toxicity study:

S.No.	Groups (Albino Rat)	Dose(Control Group)	Extract Dose (test group)	Extract Dose (test group)	Extract Dose (test group)

1	**Group I**	**Normal Saline**	**---**	**---**	**---**
2	**Group II**	**---**	**175 mg/kg**	**---**	**---**
3.	**Group III**	**---**	**---**	**500mg/kg**	**---**
4.	**Group IV**	**---**	**---**	**---**	**2000mg/kg**

3. RESULTS

Table 2: Toxicity Studies of Ethanolic Extract (Leaves) of *Koelreuteria Paniculata* in Albino Wistar Rats as per OECD guidelines 423.

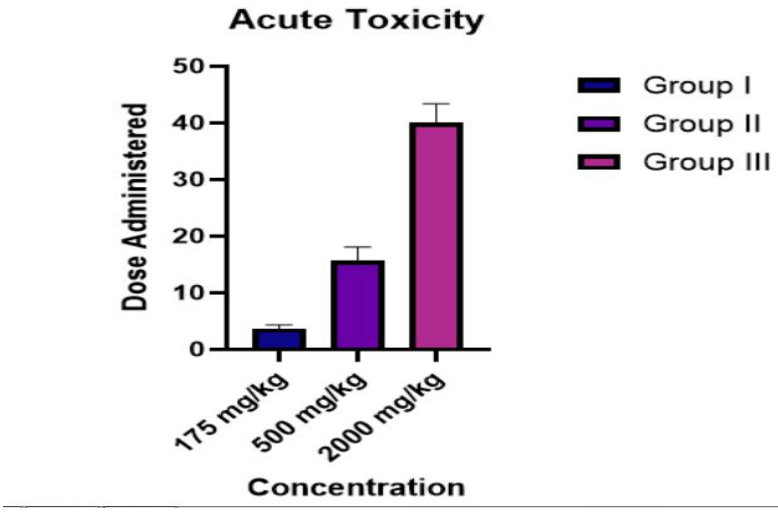
Examination	Control group	175 mg/kg	500 mg/kg	2000 mg/kg
Body weight	No change	No change	No change	No change
Temperature	No change	No change	No change	No change
Food ingestion	No change	No change	No change	No change
Urination	No change	No change	No change	No change
Skin Pigmentation disorders	No effect	No effect	No effect	No effect
Gripping strength	No change	No change	No change	No change
Grooming	Absent	Absent	Absent	Absent
Hyperactivity	Absent	Absent	Absent	Absent
Corneal response	Absent	Absent	Absent	Absent
Lacrimation	No change	No change	No change	No change
Eye coloration	No change	No change	No change	No change
Torch response	No change	No change	No change	No change
Diarrhoea	Not seen	Not seen	Not seen	Not seen
Death	Alive	Alive	Alive	Alive

Table 3: Dose administered to test animals

Groups (n=6)	Dose(mg/kg)	Ethanolic Extract of *Koelreuteria paniculata*
I	175	3.65± .67*
II	500	15.73± 2.34***
III	2000	40.07± 3.34**

Dose administered to the animals are expressed to the mean ± SD of three samples in each group. At dose level of 500 mg/kg administered orally to the animal observed better significantly ($p<0.01$,$p<0.001$)

Data represent the ethanolic extract of Koelreuteria paniculata leaves by using ANOVA analysis are not significant at 5% Represent the dose of 175 mg/kg, 500 mg/kg, 2000 mg/kg significantly ($p<0.01$,$p<0.05$,p



4. CONCLUSION

Koelreuteria paniculata also called golden rain tree native to china and others asian countries concluded that the ethanolic leaves extract of ***Koelreuteria paniculata*** has no such toxic effect. The leaves of ***koelreuteria paniculata*** was beneficial for the further clinical activities and shows potential effect against the various diseases. The pharmacological activity of the ***koelreuteria paniculata*** plant is used for the treatment of others Pharmacological disorders.

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